Effect of Acetazolamide on Larval Settlement of *Ostrea lutaria*

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(1 Text figure)

INTRODUCTION

It has been postulated that the enzyme carbonic anhydrase (CA) is involved in the formation of molluscan shell (Stolkowski, 1951; Freeman & Wilbur, 1948; Wilbur & Jodrey, 1955) as follows:

$$CO_2 + H_2O \xleftrightarrow{CA} H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^{=} \rightleftharpoons CaCO_3$$

$$CO_2 + OH^- \xleftarrow{CA} \qquad Ca^{++}$$

The strongest evidence for this is that two CA inhibitors, acetazolamide and 2-benzothiazolesulfonamide, markedly reduce the rate of deposition of calcium in the shell of *Crassostrea virginica* (Gmelin, 1791). Acetazolamide at 4.5×10^{-4} M concentration gave about 50% inhibition of calcium deposition (Wilbur & Jodrey, *op. cit.*).

In the setting process oyster larvae cement themselves permanently to a suitable substrate. These CA inhibitors were suspected of inhibiting this process and thus reducing or preventing setting. It was therefore decided to determine the effect of acetazolamide (2-benzothiazolesulfonamide not being readily available) on the setting of pediveligers of *Ostrea lutaria* (Hutton, 1873). The preliminary results reported here are surprising in that not only were the expected effects not observed but there was a significant increase in setting in the presence of acetazolamide.

METHODS AND MATERIALS

The experiment was done at sea in Foveaux Strait, the principal site of the fishery for *Ostrea lutaria* in New Zealand, to minimize the time between collection of the larvae and the start of the experiment. Numerous oysters were dredged and opened. Two oysters which were incubating late-stage pediveligers were selected for the experiment. The two groups of pediveligers were kept separate and will be referred to as Group A and Group B. Each group was suspended in 500 ml of sea water, and from each group a series of seven 50 ml aliquots was prepared. The aliquots were placed in 400 ml beakers and diluted to 250 ml. The Group A beakers contained 410 ± 36 larvae and the Group B beakers contained 480 ± 40 larvae each. In each group 6 of the beakers contained a series of dilutions of acetazolamide (Lederle) (see Figure 1) and one containing no acetazolamide was used as a control.

When larvae of *Ostrea lutaria* at the pediveliger stage are released by the female, some setting normally occurs within a few hours (Millar & Hollis, 1963). To reduce environmental variations in this experiment, no cultch shells were added and the larvae set on the walls and bottom of the beakers. After 12 hours any larvae which had not set were removed from the beakers, and the beakers were then rinsed to remove any loosely adhering larvae. The total set per beaker was then counted.

RESULTS AND DISCUSSION

The data are presented in Figure 1. There was a pronounced increase in setting with increasing amounts of added acetazolamide. At an acetazolamide concentration

of 10^{-4} M the increase in setting for both Group A and B was almost exactly 5-fold. This compares favourably with the highest increases in set accumulation of 5.61-fold obtained by Veitch & Hidu (1971) using a partially purified proteinaceous "setting factor."

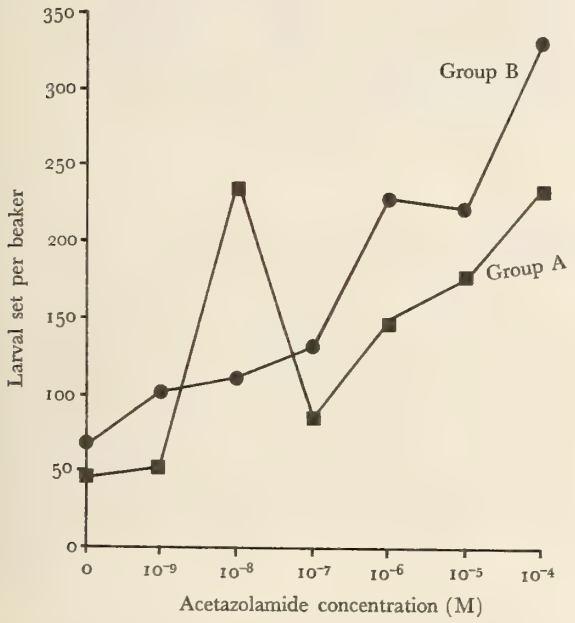

Figure 1

Number of larvae set per beaker versus molarity of acetazolamide

It is not known why there was such a large set in Group A in the beaker containing 10^{-8} M acetazolamide. The setting of oyster larvae is affected by many factors, one of which is gregarious behaviour (which may be related to the previously mentioned "setting factor") (Cole & Knight-Jones, 1949; Bayne, 1969; Hidu, 1969). It may be that several larvae set prematurely in this beaker, thus precipitating a gregarious setting effect.

The composition of larval cement is not yet fully described and furthermore it is not known whether carbonic anhydrase (CA) is involved in the process of setting. With *Crassostrea virginica*, 1×10^{-9} M acetazolamide causes just less than 50% inhibition of CA activity, and 1×10^{-8} M acetazolamide causes almost complete inhibition (Nielsen & Frieden, 1972). Therefore it seems unlikely that CA is involved in the process of setting.

The mechanism of action of acetazolamide on the setting of oyster larvae remains to be explained and will be further investigated. It may act directly to induce setting, or it may stimulate the larvae to release the proteinaceous "setting factor" of Veitch & Hidu (1971). In view of the data of Wilbur & Jodrey (1955), it must be pointed out that subsequent rearing of the larvae in the presence of acetazolamide would likely result in retarded shell growth. Therefore acetazolamide should be removed after setting has occurred.

ACKNOWLEDGMENT

This work was done at the Fisheries Research Division laboratory during the tenure of a post-doctoral fellowship from the New Zealand National Research Advisory Council, to whom I wish to express my thanks.

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